REMARKS

Claims 1-18 are currently pending in this application. With entry of this amendment, claims 1-22 will be pending; claims 1, 13, and 16 will be amended; and claims 19-22 will be added. Claims 1, 13, and 16 have been amended to set forth that the chlorinated hydantoins are present in an amount sufficient to disintegrate or remove the biofilm, flocculent bulked sludge, or bulked biologically active sludge. Support for these amendments is found on page 5, lines 31-32 ("The amount of the chlorinated hydantoin added to the aqueous medium is sufficient to disintegrate the biofilm."), page 1, line 23-27 (discussing that bulked biological sludge results from biofilm), and page 4, lines 28-30 (defining "disintegration" as including "the removal and break-up of existing biofilm and the prevention of biofilm").

New claims 19 and 20 depend from claims 1 and 19, respectively, and recite particular concentration for the chlorinated hydantoins. Support for claims 19 and 20 can be found, *e.g.*, in Table 3 on page 13 and Figures 4 and 5 showing that biofilm disintegration occurs when the concentration of chlorinated hydantoin in the aqueous medium containing the biofilm is 20 ppm (expressed as Cl_2). Support for the upper limit of the concentration of chlorinated hydantoin set forth in claim 20 can be found in claim 1 as originally filed. Support for new claim 21 can be found on page 5, lines 11-16.

Support for new claim 22 can be found on page 4, lines 5-6 of the application as filed (disclosing that the claimed method can be used in any system "subject to the growth of biofilm") and column 2, lines 14-23 and claim 8 of U.S. Patent No. 6,267,897 (filed May 4, 2000 teaching the use of plant oils to inhibit biofilms in, *e.g.*, ballast water tanks; annexed as Exhibit 1). Thus, the support for claim 22 demonstrates that, before the filing date of the present application (*i.e.*, July 20, 2005), one skilled in the art would have recognized that ballast water tanks were subject to the growth of biofilms. No new matter has been added by way of these amendments.

Applicants' Invention

The presently claimed invention is based on the unique discovery that chlorinated hydantoins can disintegrate biofilms. This property is demonstrated in Example 2 of the application as filed starting on page 9. Table 3 on page 13, discussion on page 12, lines 23-26, and Figures 4 and 5 demonstrate that a concentration of chlorinated hydantoins of 20 ppm (expressed as Cl_2) in an aqueous medium containing a biofilm disintegrates the biofilm whereas lower concentrations of the same merely control the biofilm. As discussed on page 4, line 25 to page 5, line 3, the removal of biofilm is different and more difficult compared to the control of biofilm.

Examiner's Interview on March 9, 2007

Applicants' representatives greatly appreciate Examiner Hruskoci's time on March 9, 2007 to discuss the present application. During the interview, United States Patent No. 5,662,940 to Hight *et al.* ("Hight") was discussed. Applicants' representatives asserted that Hight shows the control but not the disintegration of biofilms as presently claimed. Applicants' representatives also discussed disclosure in the specification as filed, in particular Table 3 on page 13 of the specification, which explains the difference between the control and disintegration of biofilms. The Examiner suggested that Applicants amend the claims to be consistent with the data in Table 3.

First Rejection under 35 U.S.C. §103(a)

Claims 1-6, 9-13 and 16 remain rejected under 35 U.S.C. §103(a) as obvious over Hight. According to the Examiner, Hight discloses a method of controlling microbial deposits by adding a chlorinated hydantoin to an aqueous medium. The Examiner contends that the method of Hight includes the disintegration of biofilms as presently claimed making the claimed method obvious. The Examiner also asserts that the test results provided are not commensurate with the scope of the claims.

The claims have been amended to limit the concentration of chlorinated hydantoins to an amount sufficient to disintegrate or remove biofilm or sludge. In view of these amendments,

Applicants respectfully traverse this rejection. As discussed above, the disintegration of biofilm using chlorinated hydantoins as claimed is different and more difficult compared to the control of biofilm. Hight merely discusses a "method for controlling biofouling and microorganism population levels" (emphasis added) by providing a composition containing a hypochlorite donor (such as a chlorinated hydantoin) and a bromide ion donor (*see* Abstract and col. 9, lines 30-34) in recirculating water systems such as spas (*e.g.*, *see* Example 14 starting on column 29, line 43).

Furthermore, in the previous response, Applicants argued that Hight teaches away from using hydantoin products because of their low dissolution rates, the inhibition of activity resulting from hydantoin byproducts, and their relative inactivity (*see* column 5, lines 32-64). The Examiner responded that BCDMH is a chlorinated hydantoin (*see* the last full paragraph on page 3 of the Office Action). In response, Applicants again point the Examiner's attention to the above cited Hight passage discussing the many problems with using hydantoin products, including BCDMH, and assert that Hight teaches away from using hydantoins. Instead, Hight teaches preferably using combinations of chlorinated isocyanuric acid derivatives (*see* col. 10, lines 47-63) and a bromine donor such as NaBr (*see, e.g.*, Example 1).

In light of the above amendments and arguments, Applicants respectfully request withdrawal of the rejection.

Second Rejection under 35 U.S.C. §103(a)

Claims 7, 8, 14, 15, 17 and 18 stand rejected under 35 U.S.C. § 103(a) as obvious over Hight in view of United States Patent No. 5,565,109 to Sweeny ("Sweeny"). The Examiner contends that Sweeny discloses the *in situ* formation of biocides by adding a hypochlorite and a dimethylhydantoin to an aqueous solution. According to the Examiner, a person skilled in the art could modify the method of Hight by forming the chlorinated hydantoin *in situ* in light of Sweeny as recited in the claims.

Applicants respectfully traverse the rejection. Neither Hight nor Sweeney is concerned with disintegrating biofilm using a chlorinated hydantoin as set forth in the amended claims. As discussed above, the disclosure in Hight teaches the control of biofilm. Sweeney teaches the use of halogenated hydantoins during pulp and paper manufacturing. The examples in Sweeney demonstrate the effect of halogenated hydantoins on planktonic bacteria not bacteria in biofilms. As further evidence that Sweeny does not contemplate the disintegration of biofilms, Sweeney does not differentiate between brominated and chlorinated hydantoins generally teaching the use of N-halohydantoins in a broad range of concentrations (*see* column 2, lines 30-55). As shown in the instant application (*e.g.*, in Table 3 on page 13), chlorinated hydantoins, but not bromochlorodimethylhydantoin (BCDMH), disintegrate biofilm.

Furthermore, there is no expectation in the cited prior art that the *in situ* reaction of Sweeney would yield a chlorinated hydantoin in the presence of a bromide ion as required by Hight. One skilled in the art would expect the hypochlorite, in the presence of a dimethylhydantoin, to react directly with bromide to form hypobromous acid and/or hypobromite rather than a chlorinated hydantoin because redox reactions between inorganic species tend to be much faster than substitution (*i.e.*, hydrogen-halogen substitution) reactions involving organic compounds. The hypobromite might then react with the dimethylhydantoin to form a brominated hydantoin. Only once all of the bromide is consumed one would expect excess hypochlorite to react with the hydantoin to form chloro- and possibly bromo-chlorohydantoins.

In light of the above amendments and arguments, Applicants respectfully request withdrawal of the rejection.

Conclusion

In view of the above remarks, it is respectfully requested that the application be reconsidered, all pending claims be allowed and the case be passed to issue. Since a Request for Continued Examination is submitted herewith, entry of the Response is proper. If there are any other issues remaining which the Examiner believes could be resolved through a Supplemental

Response or an Examiner's Amendment, the Examiner is respectfully requested to contact the undersigned at the telephone number indicated below.

Dated: April 5, 2007

Respectfully submitted,

By _____

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EXHIBIT 1

US006267897B1

(12) **United States Patent**
Robertson et al.

(10) **Patent No.: US 6,267,897 B1**
(45) **Date of Patent: Jul. 31, 2001**

(54) **METHOD OF INHIBITING BIOFILM FORMATION IN COMMERCIAL AND INDUSTRIAL WATER SYSTEMS**

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(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 0 days.

(21) Appl. No.: **09/564,244**

(22) Filed: **May 4, 2000**

(51) **Int. Cl.**[7] **C02F 1/50**
(52) **U.S. Cl.** **210/764**; 252/180
(58) **Field of Search** 210/764; 252/175, 252/180

(56) **References Cited**

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(57) **ABSTRACT**

Biofilm formation is inhibited in commercial and industrial water systems through the addition of at least one plant oil.

8 Claims, No Drawings

METHOD OF INHIBITING BIOFILM FORMATION IN COMMERCIAL AND INDUSTRIAL WATER SYSTEMS

FIELD OF THE INVENTION

This invention relates generally to the field of water treatment technologies and, more particularly, to a method of inhibiting biofilm formation in commercial and industrial water systems.

BACKGROUND OF THE INVENTION

Biofouling has always been problematic in commercial and industrial water systems, such as cooling tower waters and air washers, because it can adversely affect heat transfer efficiency and fluid frictional resistance, thereby subsequently reducing production rates. Biofouling is also a problem in pulp and paper mill systems because the growth of microorganisms in papermachine fluids can adversely affect finished paper products, thereby requiring the papermachine to be shut down, resulting in the loss of productivity brought on by the down time of the machine. Furthermore, biofouling plays an important role in microbiologically influenced corrosion.

The presence of microorganisms in commercial and industrial waters cannot be totally eliminated, even with the excessive use of chemical biocides. The most common way to control biofouling is through the application of toxic chemical biocides such as chlorine, bromine, isothiazolones, glutaraldehyde or other antimicrobials. These biocides are added in an attempt to kill both planktonic and attached microorganisms.

Some microorganisms attach to inert surfaces forming aggregates with a complex matrix consisting of extracellular polymeric substances (EPS). This consortium of attached microorganisms and the associated EPS is commonly referred to as a biofilm. Biocides have difficulty penetrating biofilms and removing them from surfaces. Although excessive biocide dosages may be able to control biofouling, such use is costly and the presence of biocides in effluent waters is usually environmentally unacceptable.

Accordingly, it would be desirable to provide a method of inhibiting biofilm formation in commercial and industrial water systems which utilizes a low-cost, non-biocidal substance.

SUMMARY OF THE INVENTION

The method of the invention calls for adding one or more plant oils to a commercial or industrial water system. The addition of plant oil efficiently and effectively inhibits biofilm formation in commercial and industrial water systems. Moreover, the inventive method is economically appealing and environmentally acceptable because plant oils are low in cost and non-biocidal.

DETAILED DESCRIPTION OF THE INVENTION

The present invention is directed to a method of inhibiting biofilm formation in commercial and industrial water systems. In accordance with this invention, one or more plant oils are added to the commercial or industrial water system.

"Plant oils" (which are also known in the art as "natural oils" or "essential oils") are generally defined as volatile oils obtained from plants which possess the odor and other characteristics of the plant. The plant oils that may be used in the practice of this invention include eucalyptus, cinnamon, retsin, tea tree, clove, camphor, pine, spruce, neem, peppermint, spearmint, wintergreen, lime, orange, grapefruit, mandarin, lemongrass and citronella oils, as well as mixtures thereof. Eucalyptus oil and cinnamon oil are the most preferred plant oils.

The plant oils can be added to the commercial or industrial water system by any conventional method at a concentration which effectively inhibits biofilm formation. It is preferred that the amount of plant oil which is added to the commercial or industrial water system be in the range of about 1 ppm to about 10,000 ppm. More preferably, the amount of plant oil is from about 1 ppm to about 5,000 ppm, with about 1 ppm to about 250 ppm being most preferred.

The commercial and industrial water systems to which the plant oils may be added to inhibit biofilm formation include cooling waters; food, beverage and industrial process waters; pulp and paper mill systems; brewery pasteurizers; sweetwater systems; air washer systems; oil field drilling fluids and muds; petroleum recovery processes; industrial lubricants; cutting fluids; heat transfer systems; gas scrubber systems; latex systems; clay and pigment systems; decorative fountains; water intake pipes; ballast water tanks; and ship reservoirs, among others.

EXAMPLE

The following example is intended to be illustrative of the present invention and to teach one of ordinary skill how to make and use the invention. The example is not intended to limit the invention or its protection in any way.

A jar test was conducted to demonstrate the ability of the plant oils to interfere with the attachment of filamentous bacteria to surfaces. The plant oils of the invention were applied to paper machine fluids to prevent the attachment of microorganisms to machine surfaces and thereby prevent contamination of the machine by filamentous and other bacteria. Furthermore, by preventing the attachment of the biofilm, the plant oils also helped prevent contamination by wood fibers and fillers.

Sphaerotilus natans (a filamentous, slime-forming bacterium common to paper mills) was grown in a modified nutrient medium designed to promote biofilm formation. The uniform inoculum was harvested and aliquots were frozen and stored at −70° C. until needed.

Eight-ounce flush glass jars were rinsed once in acetone and twice in deionized water to remove any surface contaminants. The jars were autoclaved at 121° C. for 30 minutes. Predetermined volumes of the plant oils (cinnamon oil, eucalyptus oil and tea tree oil) were added directly to the jars.

50 mL of sterile medium were added to each jar and 100 μl of the thawed *Sphaerotilus natans* culture were added to each jar. The jars were placed into a New Brunswick Series 25 orbital shaker at 35° C. at 210 rpm. After 48 hours of incubation, the biofilms which attached and formed at the base of the jars were measured for diameter in centimeters and rated for vigor on a scale of 0 to +4. As used herein, "vigor" is defined as the visual characterization of the thickness and tenacity of the biofilm. A modified geometric mean was calculated for each jar by taking the square root of the diameter (in centimeters) multiplied by the vigor. Each experiment was set up with a minimum of three jars per concentration and treatment chemical. The average of all modified geometric means calculated for a specific plant oil and dosage was then averaged and listed in Table 1.

The results of the treatments are shown below in Table 1. The data illustrates that with cinnamon oil, the modified

geometric mean of diameter and vigor at a 25 ppm dose is 1.6, which is significantly better than the ethylene oxide/propylene oxide (EO/PO) copolymer (Nalco product N-7611) in preventing microbial attachment to surfaces since a lower response number indicates a more active compound. EO/PO copolymers are currently used in industry to inhibit biofilm formation.

Table 1 also shows eucalyptus oil and tea tree oil at 100 ppm to perform better than the EO/PO copolymer at inhibiting biofilm formation.

TABLE 1

Treatment		Average Modified Geometric Mean	Average Diameter (cm)
Eucalyptus oil			
	@ 250 ppm	1.71	1.47
	@ 200 ppm	1.98	2.18
	@ 100 ppm	2.5	3.08
	@ 20 ppm	2.87	3.63
Cinnamon Oil			
	@ 25 ppm	1.6	2.38
	@ 10 ppm	3.07	3.77
	@ 5 ppm	3.21	5
Tea Tree Oil			
	@ 100 ppm	3.16	2.55
	@ 20 ppm	4.18	6
Ethylene oxide/propylene oxide copolymer			
	@ 30 ppm	3	3.2
	@ 15 ppm	3.4	4.7
	@ 5 ppm	4.7	5.8
untreated		4.7	6

While the present invention is described above in connection with preferred or illustrative embodiments, these embodiments are not intended to be exhaustive or limiting of the invention. Rather, the invention is intended to cover all alternatives, modifications and equivalents included within its spirit and scope, as defined by the appended claims.

What is claimed is:

1. A method of inhibiting biofilm formation in commercial and industrial water systems comprising the step of adding thereto an effective inhibiting amount of an agent consisting essentially of at least one plant oil.

2. The method of claim **1** wherein the plant oil is selected from the group consisting of eucalyptus, cinnamon, retsin, tea tree, clove, camphor, pine, spruce, neem, peppermint, spearmint, wintergreen, lime, orange, grapefruit, mandarin, lemongrass and citronella oils, and mixtures thereof.

3. The method of claim **1** wherein the plant oil is eucalyptus oil.

4. The method of claim **1** wherein the plant oil is cinnamon oil.

5. The method of claim **1** wherein the plant oil is added to the water system in an amount from about 1 ppm to about 10,000 ppm.

6. The method of claim **1** wherein the plant oil is added to the water system in an amount from about 1 ppm to about 5,000 ppm.

7. The method of claim **1** wherein the plant oil is added to the water system in an amount from about 1 ppm to about 250 ppm.

8. The method of claim **1** wherein the commercial and industrial water systems are selected from the group consisting of cooling waters; food, beverage and industrial process waters; pulp and paper mill systems; brewery pasteurizers; sweetwater systems; air washer systems; oil field drilling fluids and muds; petroleum recovery processes; industrial lubricants; cutting fluids; heat transfer systems; gas scrubber systems; latex systems; clay and pigment systems; decorative fountains; water intake pipes; ballast water tanks; and ship reservoirs.

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